Observations of clonal cultures of Euglyphidae (Rhizopoda, Protozoa)

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Introduction

The identification of small siliceous testate amoebae is often difficult due to three main factors, observations are made at the limits of the optical microscope, scarcity of specimens and inadequate descriptions. The introduction of scanning-electron microscopy for detailed surface examination has reduced the first of these obstacles, as this facility permits more accurate descriptions to be made of the shells of these animals. However, matching these more detailed descriptions with existing reports is often complicated as slight differences of shell structure have frequently prompted authors to propose new specific names, or new 'varieties' or new 'forms'. The second factor can also be reduced by establishing clonal cultures in the laboratory; additionally such cultures enable one to study biology and variation in morphology. Previous reports (Hedley & Ogden, 1973, 1974; Hedley *et al.*, 1974) dealt with the biology of four species, namely *Euglypha acanthopora* (Ehrenberg, 1841), *E. rotunda* Wailes, 1911, *E. strigosa* (Ehrenberg, 1871) and *Trinema lineare* Penard, 1890.

The present account describes the shell morphology and biology of four further species, based on clonal cultures, together with the redescription of a previous clone under a new specific name.

Systematics

The genera *Euglypha* and *Assulina* belong to the family Euglyphidae—the classification adopted here is that proposed by Levine *et al.*, 1980 and Loeblich and Tappan, 1964:

Superclass	RHIZOPODA	Von Siebold, 1845
Class	FILOSEA	Leidy, 1879
Order	GROMIIDA	Claparede and Lachmann, 1859
Superfamily	EUGLYPHACEA	Loeblich and Tappan, 1961
Family	EUGLYPHIDAE	Wallich, 1864
		shell hyaline, symmetrical, elongate, composed of rounded siliceous plates, aperture rounded or elongate; one nucleus.

Euglypha acanthophora—In identifying specimens from a clonal culture as *E. acanthophora* Hedley *et al.* (1974) accepted the reported variation attributed to this species, although with some reservation because the extent of the variations was not substantiated by our observations of other *Euglypha* species in culture. These differences mainly concerned the shape and position of the siliceous spines and the shape of the body plates, which are illustrated in Leidy's superb figures (Leidy, 1879) of *E. alveolata*—a species accepted by both Cash *et al.*, (1915) and Penard (in Cash *et al.*) as a synonym of *E. acanthophora*—showing that the siliceous spines project either distinctly from the sides of the body or discretely from the aboral extremity, and that there are distinct tooth-like projections on the posterior edge of the body plates. Penard (1902) proposed two varieties to accommodate specimens with

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varying spines, and these were accepted by Cash *et al.,* (1915) who also suggested that intermediate forms existed. The extent of these variations is given by Decloitre (1962) in a review of the genus *Euglypha,* which lists seven varieties of *E. acanthophora.*

The limitation of our research was such that it was expedient at that time (Hedley *et al.,* 1974) to consider our clonal culture to be representative of *E. acanthophora.* The problem of designated names now arises with the establishment of another distinct clonal culture within the heterogenous description of *E. acanthophora.* It appears that the most recent culture is more closely allied to the description of *E. acanthophora,* whilst the earlier culture (Hedley *et al.,* 1974) fits the description of *E. a.* var. *brevispina* given by Cash *et al.,* (1915). However, this latter description does not agree with the original (Penard, 1902) as discussed on p. 140, so to avoid confusion with these variety names it is proposed that a new specific name, *E. cashii,* be given to the earlier clonal culture.

Materials and methods

Information on the source of each species is given with the taxonomic description.

The animals were obtained from crude cultures made by isolating small portions of each sample placed in a small plastic container and covered with a shallow layer of the culture liquid. Agnotobiotic cultures were kept at room temperature (18–20°C), in similar containers, on a thin layer of agar agar (1 per cent in distilled water) with a sterilised wheat grain added prior to setting and covered by a shallow layer of culture medium. The culture medium was a 5 per cent solution of soil extract plus added nutrient salts (see Hedley & Ogden, 1973). Clonal cultures were established by isolating single active animals, and one clone has been used subsequently to produce the working cultures of each species. Subcultures made at 2–3 week intervals are adequate to maintain active animals.

Live specimens were examined by optical microscopy using both phase-contrast and brightfield illumination. Specimens for scanning electron microscopy were cleaned by transferring them through several changes of distilled water. Then manipulated using a single-hair brush onto small drops of Araldite adhesive on a small cover slip, the cover slip having been previously cleaned and dried. The exception to this procedure were specimens of *E. dickensii* which proved to be fragile and collapsed using this technique. Alternatively they were fixed in 3 per cent glutaraldehyde in O·1M cacodylic acid buffer, rinsed in the buffer solution and several times in distilled water, then specimens in distilled water were micropipetted onto a clean cover slip and allowed to dry. Although some specimens still collapsed, the majority retained their natural shape. The prepared cover slips were fastened to aluminium stereoscan stubs with Araldite, prior to being coated evenly with a thin layer of conducting metal. The stubs were examined on a Cambridge Stereoscan 180 operating at 10kV and the results recorded on Ilford HP5 film.

Morphology

Euglypha acanthophora (Ehrenberg, 1841)

syn.   *Euglypha alveolata* (Dujardin, 1841)—in Leidy, 1879, pl. 35, figs 3, 7, 15–18
        Euglypha brachiata Penard, 1902 (Non Leidy, 1878)
        Euglypha brachiata var. *flexulosa* Penard, 1902
        Euglypha brachiata var. *brevispina* Penard, 1902 (Non Cash *et al.,* 1915)
        Euglypha armata Wailes and Penard, 1911
        Euglypha crenulata var. *minor* Wailes, 1912
        Euglypha acanthophora var. *dorsalis* Schönborn, 1962

This species was isolated from a sample of *Sphagnum* moss collected at Holmsley, near Burley, New Forest, Hampshire, in March 1979.
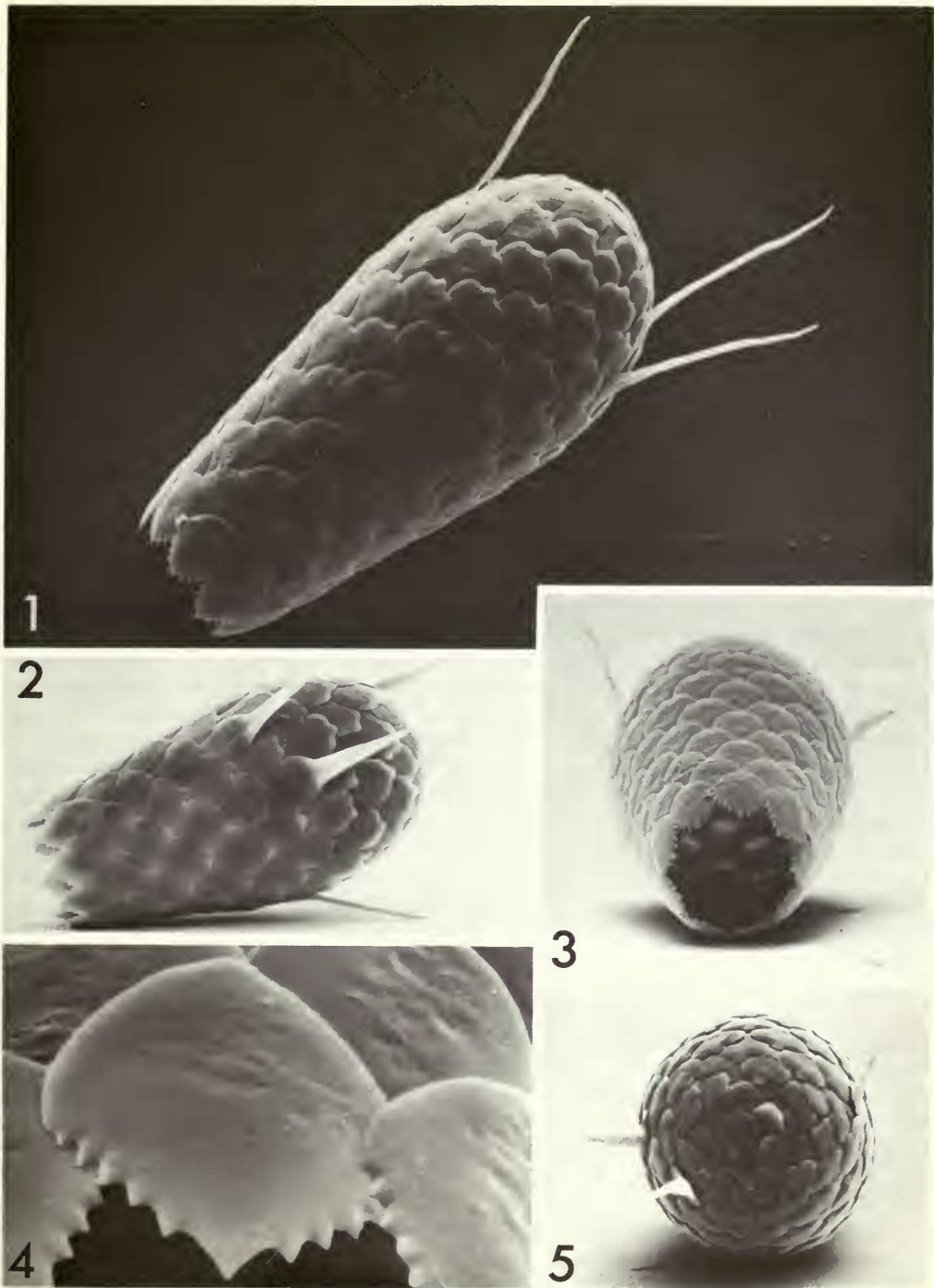
Figs 1–5 *Euglypha acanthophora*: Figs 1 & 2, lateral views to illustrate the distribution of body and elongated plates × 1250 & × 970; Fig. 3, view of aperture with nine apertural plates × 1170; Fig. 4, single apertural plate, note the slight thickening on the dentate margin × 7500; Fig. 5, terminal or aboral view showing the distribution of oval body plates × 1200.

Table 1 Range of measurements (in μm).

|		length	breadth	depth	diameter of aperture
Euglypha acanthophora	(35)	54·4–68·6	26·4–33·8		12·8–16·5
Euglypha cashii	a.* (53)	65·1–84	33·6–42		16·8–25·2
	b. (10)	67–88·9	33·9–39·8		15·3–19·2
Euglypha compressa	(31)	60·8–74·7	36·2–46·4	19·7–25·6	12·1–17·7
Euglypha dickensii	(32)	35·3–53·5	20·7–34·4	14·1–18·9	9·1–14·7
Assulina muscorum	(33)	33·7–54	25·2–36·6	15·4–19·6	7·7–14·1

* measurements from Hedley *et al.,* 1974; bracketed figures indicate the number of specimens measured.
  a. Clone from Hertfordshire, b. clone from Wales.

DESCRIPTION. The shell is elliptical or ovoid, circular in cross section, composed of about one hundred and thirty body plates and up to six elongated plates or spines (Table 1, Figs 1 & 2). The aperture is terminal, circular and surrounded by between eight and eleven, usually evenly spaced, denticulate apertural plates (Fig. 3). Each apertural plate is roughly oval, between 8·1–10·4 μm in length and 6·3–7·8 μm in width, often both the denticular and the opposite margin are pointed (Fig. 4). The dentate edge is barely thickened on the inner surface around the median tooth and the five smaller lateral teeth positioned on each side (Fig. 4). Denticular margins are also seen on the second circle of plates surrounding the aperture. The body plates are arranged in alternate, longitudinal rows, and range from 7·5–9·8 μm in length and 6·3–8·1 μm in width. Usually the posterior margin of these roughly oval plates has a median rounded projection, this appears to be more pronounced when the plate has an angular shape (Fig. 6). Body plates appear to vary in shape depending on their position, the pointed posterior margin of the plate becoming more pronounced in progression down the length of the body (Fig. 1), only to be rounded in some instances at the aboral extremity (Fig. 5). The elongated plates are dagger shaped, ranging in length from 18–30 μm, and usually project from the sides of the body at an acute angle (Figs 1 & 2). They may be positioned anywhere in the posterior half of the shell, but are only rarely seen projecting from the extremity. The elongated portion of these plates is thin, hence their brightness when bombarded by electrons in the scanning electron microscope (Figs 7 & 8), and vary markedly in breadth which in turn alters the degree of tapering. It would appear that narrow and long spines are more flexible (Fig. 7), whilst the broader and shorter spines are more robust (Fig. 8).

DISCUSSION. Both Cash and Penard described *E. acanthophora* with long thin spines and *E. acanthophora* var. *brevispina* with short stout spines. There is good agreement between the diagrams of *E. acanthophora* given by Cash (1915: pl. 35, fig. 2), Leidy (1879 pl. 35 figs 3, 7, 15–18) and Penard (1902 p. 505 and in Wailes & Penard, 1911: pl. 3, fig. 16), but not between the diagrams for *E. a.* var. *brevispina,* those of Cash and Leidy (1915: pl. 33, figs 3, 5; 1879 pl. 35, figs 1, 2, 4) being quite different from the original Penard figures (1902 p. 505 figs 6–10). Whereas the spines shown by the former authors are pointed, Penard's are blunt and appear to have broken or ragged edges. As Penard's two varieties—*brevispina* and *flexulosa*—differ only in having as their names imply either truncate or flexous spines, features which are considered to be artifacts from observations on clonal cultures reported here, they are listed as synonyms of *E. acanthophora.*

Although the apertural plates in the above mentioned descriptions are similar, there are differences in the distribution and shape of the body plates. For example, Leidy (1879) noted that there were two rows of denticulate plates surrounding the aperture and that the body plates had small projections from their posterior margins. Both Cash and Penard agree with
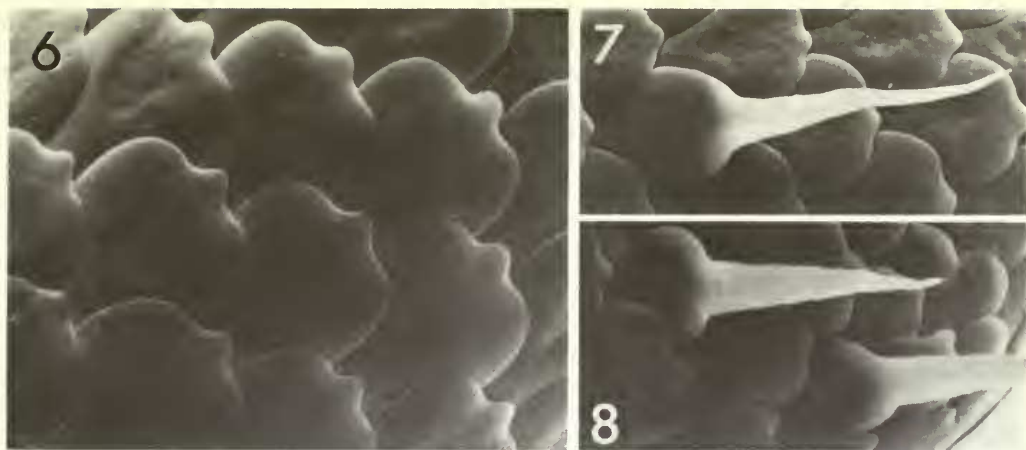
Figs 6–8   *Euglypha acanthophora*: Fig. 6, area of shell surface showing typical body plates × 3300;
Fig. 7, 'dagger-shaped' elongated plate, a narrow and flexible type × 2500; Fig. 8, a stout and
robust type of elongated plate × 2700.

the two rows of denticulate plates, but neither commented on the body plates–except for a
reference in the generic discussion (Cash *et al.,* 1915: p. 5) naming just three species with
'scutiform' plates : *E. scutigera* Penard, 1911; *E. aspera* Penard, 1899 and *E. crenulata*
Wailes, 1911—nevertheless they synonymize Leidy's description with *E. acanthophora.*
Wailes (1912) in describing a new variety, *E. crenulata var. minor,* noted that it was only
distinguished from *E. armata* Wailes & Penard, 1911 (a synonym of *E. acanthophora*
proposed by Wailes in Cash *et al.,* 1915) by the shape of the body plates.

Of the eleven varieties listed by Decloitre (1962, 1976), two have already been mentioned,
E. a. var. *brevispina* and *E. a.* var. *flexulosa*; four appear to be similar to *E. acanthophora* as
described here, these are *E. a.* var. *cylindracea* (Playfair, 1918), *E. a.* var. *dorsalis* Schönborn,
1962, *E. a.* var. *fantastica* Decloitre, 1965 and *E. a.* var. *longispina* Decloitre, 1969; two, *E.
a.* var. *equeis* Decloitre, 1956 and *E. a.* var. *heterospina* Decloitre, 1949, have discrete
arrangements of spines and may represent distinct species; and the remaining three are
considered later on p. 143. The four similar varieties differ in size (*cylindracea*), slight
deflection of the aperture (*dorsalis*), the shape of the spines (*fantastica*), and the number of
spines (*longispina*). Specimens with misaligned apertures are not uncommon in clonal
culture, and *E. a.* var. *dorsalis* is therefore considered to be a synonym of *E. acanthophora.*
The differences of the three remaining varieties may also be artificial, but it is not possible to
comment further on these.

Euglypha cashii nom. nov.

syn.   *Euglypha acanthophora* var *brevispina*—in Cash *et al.,* 1915
       Euglypha alveolata—in Leidy, 1879: pl. 35, figs 1, 2, 4
       Euglypha alveolata var. *cirrata* Wailes, 1912
       Euglypha acanthophora var. *deflanderi* Decloitre, 1956
       Euglypha acanthophora var. *elegans* Stepanek, 1963
       Euglypha acanthophora—in Hedley *et al.,* 1974
       Euglypha acanthophora—in Ogden & Hedley, 1980

Two clones of this species have been isolated, the first from sewage sludge from the Maple
Lodge Works of the Colne Valley Sewage Board, Hertfordshire, in December, 1972 and the
second from a sample of *Sphagnum* moss from Myndd Hiraethog, Denbigh, Clwyd, North
Wales, in August, 1980.
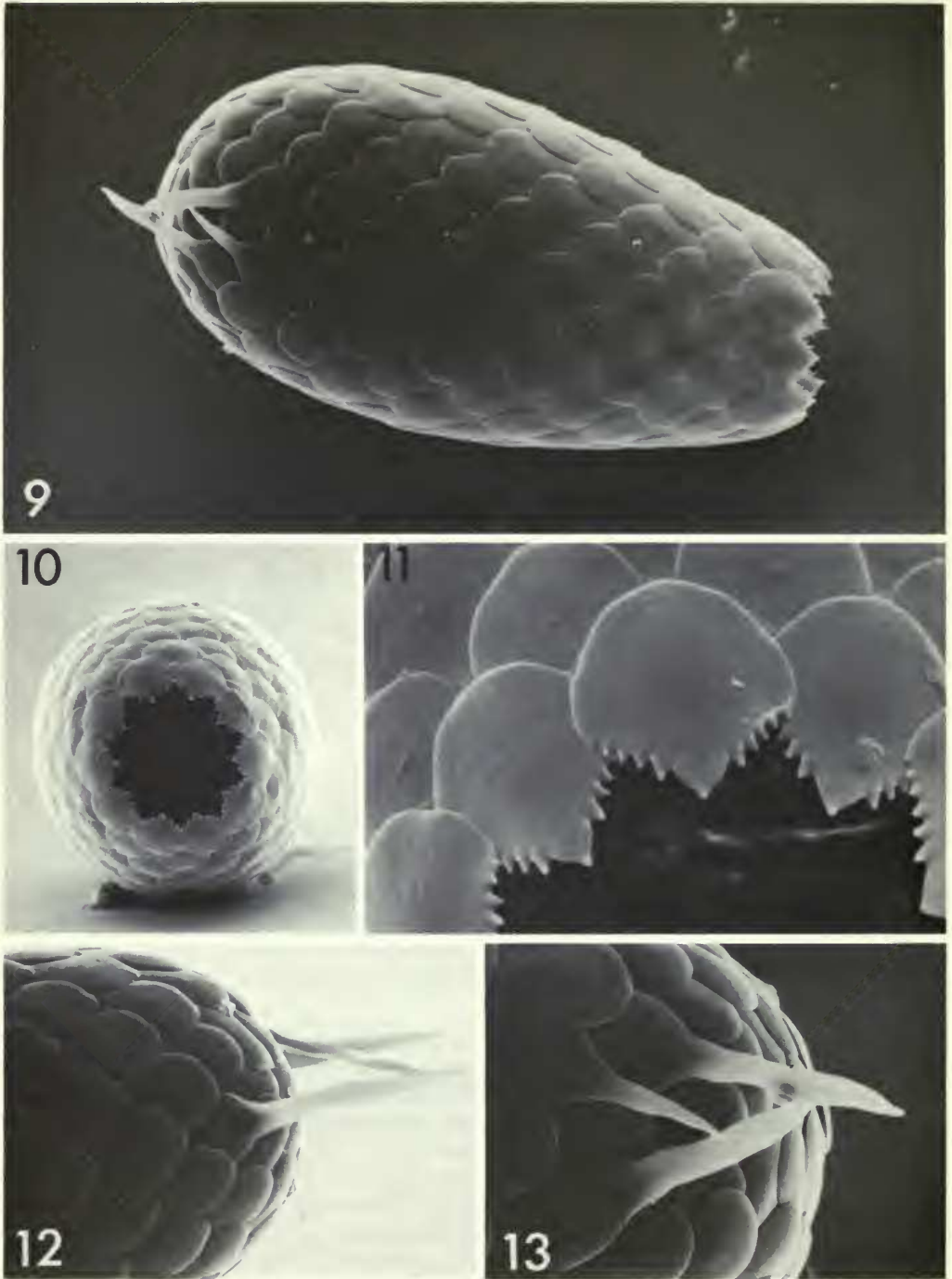
Figs 9–13   *Euglypha cashii*: Fig. 9, lateral view showing the distribution of body and elongated plates × 1250; Fig. 10, aperture with ten evenly distributed apertural plates × 1100; Fig. 11, detail of apertural plates × 4400; Fig. 12, elongated plates projecting from aboral extremity × 1800; Fig. 13, elongated plates following the curvature of the shell × 2400.

DESCRIPTION. The shell is elliptical or ovoid, circular in cross section, composed of about two hundred body plates and up to six elongated plates (Table 1, Fig. 9). The aperture is terminal, circular and surrounded by between ten and thirteen evenly spaced denticulate apertural plates (Fig. 10). Each apertural plate is roughly oval, varying from 8·1–12·5 µm in length and 7·2–11·5 µm in width. The dentate edge is slightly thickened and carries a median tooth with either four or five smaller lateral teeth on each side (Fig. 11). Specimens are sometimes seen with the average number of plates surrounding the aperture but with either one or two being dentate body plates, similar to those shown in Fig. 1a of Hedley *et al.* (1974), instead of dentate apertural plates. The body plates are arranged in alternate longitudinal rows and range from 10·9–12 µm in length and 7·9–9·1 µm in width. Each oval body plate has a median rounded projection on the posterior margin, these projections are not as pronounced as in *E. acanthophora* (compare Figs 1 & 9). The elongated plates project slightly from the aboral extremity, or closely follow the outline of the shell in the aboral region, they range from 19·6–29·2 µm in length and 6·1–7·6 µm in width (Figs 12 & 13).

DISCUSSION. The description of this species is in good agreement with that given by Cash *et al.* (1915: pl. 33, figs 3 & 5) for *E. a.* var. *brevispina.* As there is some disparity between these two descriptions and the original description of the variety *brevispina* given by Penard (1902) (see also p. 140), in addition to the variety name *brevispina* being used with other species of *Euglypha,* it was considered that a new specific name would eliminate future confusion of these species. *E. cashii* is distinguished from *E. acanthophora* by size, shape of body and elongate plates, but mainly by the positioning of the elongated plates.

The three varieties of *E. acanthophora* listed by Decloitre (1962, 1976) which are similar to *E. cashii* are *E. a.* var. *cirrata*: *E. a.* var. *deflanderi* and *E. a.* var. *elegans,* they apparently differ only in the deflection of the posterior spines or elongated plates to the shell body. Such features have been seen in clonal culture as a normal variation, and these varieties are therefore considered to be synonyms of *E. cashii.* Another species, *E. tiscia* Gal, 1969, listed by Decloitre (1976), although similar to *E. cashii* is considered to represent a valid species because of its larger dimensions.

ETYMOLOGY. This species is named after Mr James Cash, who contributed so much to the taxonomy of testate amoebae at the beginning of this century.

Euglypha compressa Carter, 1864

This species was isolated from a sample of *Sphagnum* moss, collected at Subberthwaite, Broughton in Furness, Cumbria, in July, 1978.

DESCRIPTION. The shell is ovoid, laterally compressed and composed of about two hundred body plates and thirty spines (Table 1, Fig. 14). The aperture is terminal, circular or elliptical and surrounded by between eleven and fifteen, evenly spaced, denticulate apertural plates (Fig. 15). Each apertural plate is oval, between 6·2–7·1 µm in length and 4·6–5·4 µm in width. The dentate edge is distally thickened around the large median tooth, but this thickening tapers evenly outwards to the three smaller teeth positioned on each side and equates with the normal shell thickness close to the last tooth (Fig. 16). The body plates are arranged in sequence and range in size from 7·4–8·2 µm in length and 3·4–4·4 µm in width. They are roughly oval and often characterised by their hexagonal appearance, this latter feature is variable but the squared nature of the narrow margins is usually distinctive (Fig. 17). The spines are positioned along the lateral margins from about the mid-body position to the aboral extremity (Fig. 15). They are sometimes in pairs and usually alternate when viewed laterally, one pointing upwards and the next downwards along the length of the body (Figs 14 & 15). Each spine has a narrow base from which it tapers to its widest point about a quarter of the total spine length, then it tapers gradually over the remaining three-quarters to the terminal point (Fig. 18).

DISCUSSION. The only recorded variety of this species is *E. compressa* f. *glabra* Cash *et al.,*
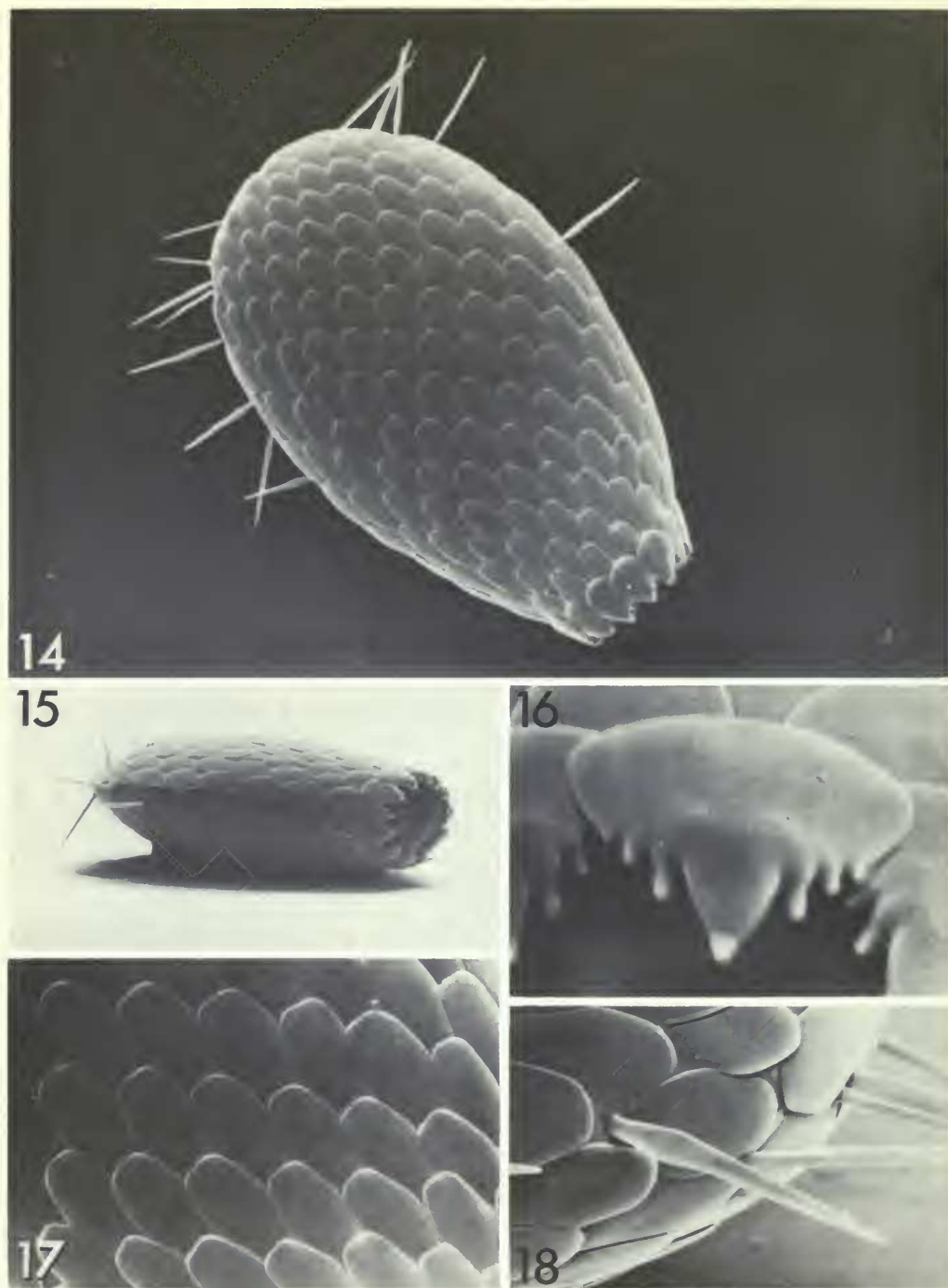
Figs 14–18 *Euglypha compressa*: Fig. 14, lateral view showing the distribution of body plates and spines × 1250; Fig. 15, latero-apertural view to illustrate the circular aperture and the positioning of the spines × 910; Fig. 16, detail of single apertural plate, note the thickening around the dentate margin × 9500; Fig. 17, arrangement of typical body plates × 2350; Fig. 18, illustration of spine projecting from organic cement matrix between two adjacent body plates × 4400.

1915, a form without spines. However, variation in the shape and size of the siliceous spines was also reported by Cash *et al.* for *E. compressa,* and such differences were considered to be a normal feature. This may not now prove to be the case, if, as the information derived from clonal cultures in the present work shows that differences in spine positioning is specific, then it would appear to follow that structural differences of spine construction may also be specific. The spines described here are similar to those reported by de Graaf (1956) and Brown (1910), but they are different from those described by Ogden & Hedley (1980). In the latter report the spines are stout at the base, concave, and taper evenly to a point (see Pl. 78D, Ogden & Hedley, 1980). Both types of spine are here considered to represent *E. compressa,* which is distinguished by the compressed shell and lateral position of the spines, but further work on clonal cultures may establish spine shape as a specific character.

Euglypha dickensii sp. nov.

This species was isolated from a sample of damp moss taken from underneath sweet chestnut trees at Cobham woods, Rochester, Kent in February, 1974.

DESCRIPTION. The shell is ovoid, laterally compressed and composed of about two hundred and eighty elongate body plates (Table 1, Figs 19 & 20). The aperture is terminal, oval or circular, and surrounded by between eleven and fifteen, evenly spaced, denticulate apertural plates (Fig. 21). In a few specimens additional apertural plates are seen in the second circle of plates around the aperture. Each apertural plate is roughly circular, from 5·4–6·4 µm in length and 3·8–4·4 µm in width. The dentate edge has a large, thick, distinctly curved, median process with a terminal pointed tooth, this is flanked on each side by a medium outward facing tooth and one or two smaller teeth (Figs 22 & 24). The denticular thickening equates with the normal shell thickness at the position of the small teeth. The body plates are elongate, ranging from 5·1–6·2 µm in length and 1·6–2·5 µm in width, and are arranged in alternate longitudinal rows. Around the mid-body region there are some randomly distributed pointed body plates (Fig. 23). These pointed plates vary from normal plates with a small sharp spike, about 6·9 µm long, to tapered spines about twice the length, 9·1 µm, of a normal body plate. Although there is variation in the dimensions of these pointed body plates, their presence in a mid-body position is a reasonably stable feature.

DISCUSSION. The species described here is similar to three species previously reported from soil samples, namely *E. capsiosa* Couteaux, 1978, *E. cuspidata* Bonnet & Thomas, 1960 and *E. simplex* Decloitre, 1965. It has similar dimensions to *E. simplex* but differs in the shape of the apertural plates, which in the latter species have a distinct diamond-shape (see Couteaux *et al.,* 1979). Both *E. capsiosa* and *E. cuspidata* are smaller species with fewer body and apertural plates, and again differ in the shape of the apertural plates. *E. dickensii* is distinct in size, dentation of the apertural plates and the presence of pointed body plates in the mid-body region.

ETYMOLOGY. As this species was found in the countryside frequented by Charles Dickens, the nineteenth century author, and subsequently featured in many of his novels, it is named in his honour.

Assulina muscorum Greef, 1888

This species was isolated from a sample of dry moss on soil, collected at Rolestone Farm, Banwell, Somerset in February, 1974.

DESCRIPTION. The shell is ovoid, laterally compressed and composed of about two hundred shell plates (Figs 25 & 26). The aperture is terminal and surrounded by between ten and fourteen shell plates arranged in a rather irregular manner, most with their minor axis bordering the opening but often a few have their major axis (Figs 27 & 28). The opening is edged with a thin band of organic cement, this band is frequently thickened on the tips of some plates to form tooth-like projections (Fig. 28). The shell plates are oval, ranging from 5·8–6·8 µm in length and 2·5–3·1 µm in width. They are usually arranged in alternate,
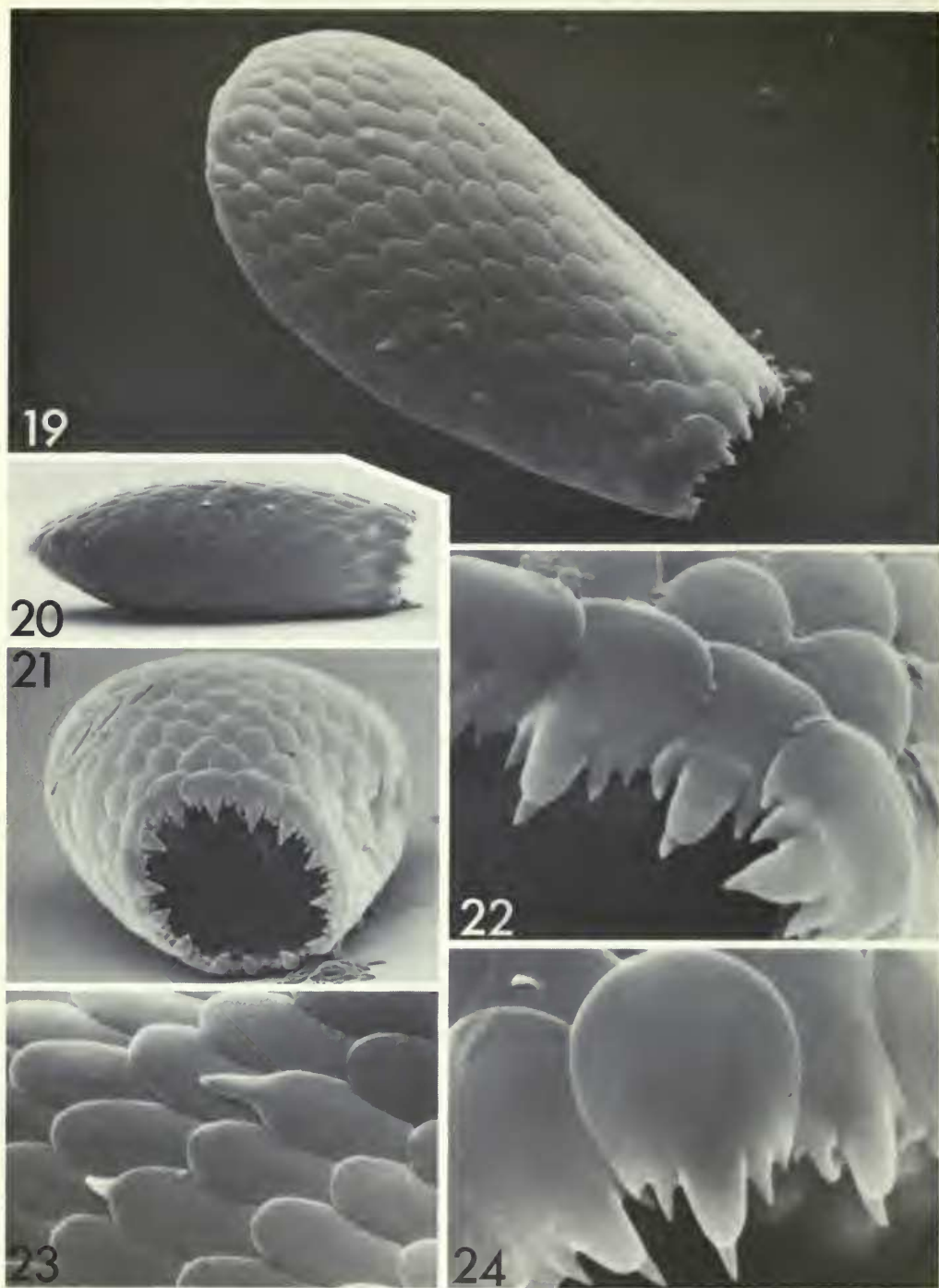
Figs 19–24 *Euglypha dickensii*: Fig. 19, lateral view showing the distribution of body plates × 1970; Fig. 20, view to illustrate the lateral compression × 1300; Fig 21, aperture with fourteen apertural plates × 2400; Fig. 22, view showing the overlapping of the apertural plates and the thickness of the median tooth × 7800; Fig. 23, portion of shell surface with two pointed body plates × 4600; Fig. 24, circular apertural plate with typical dentate margin × 11000.
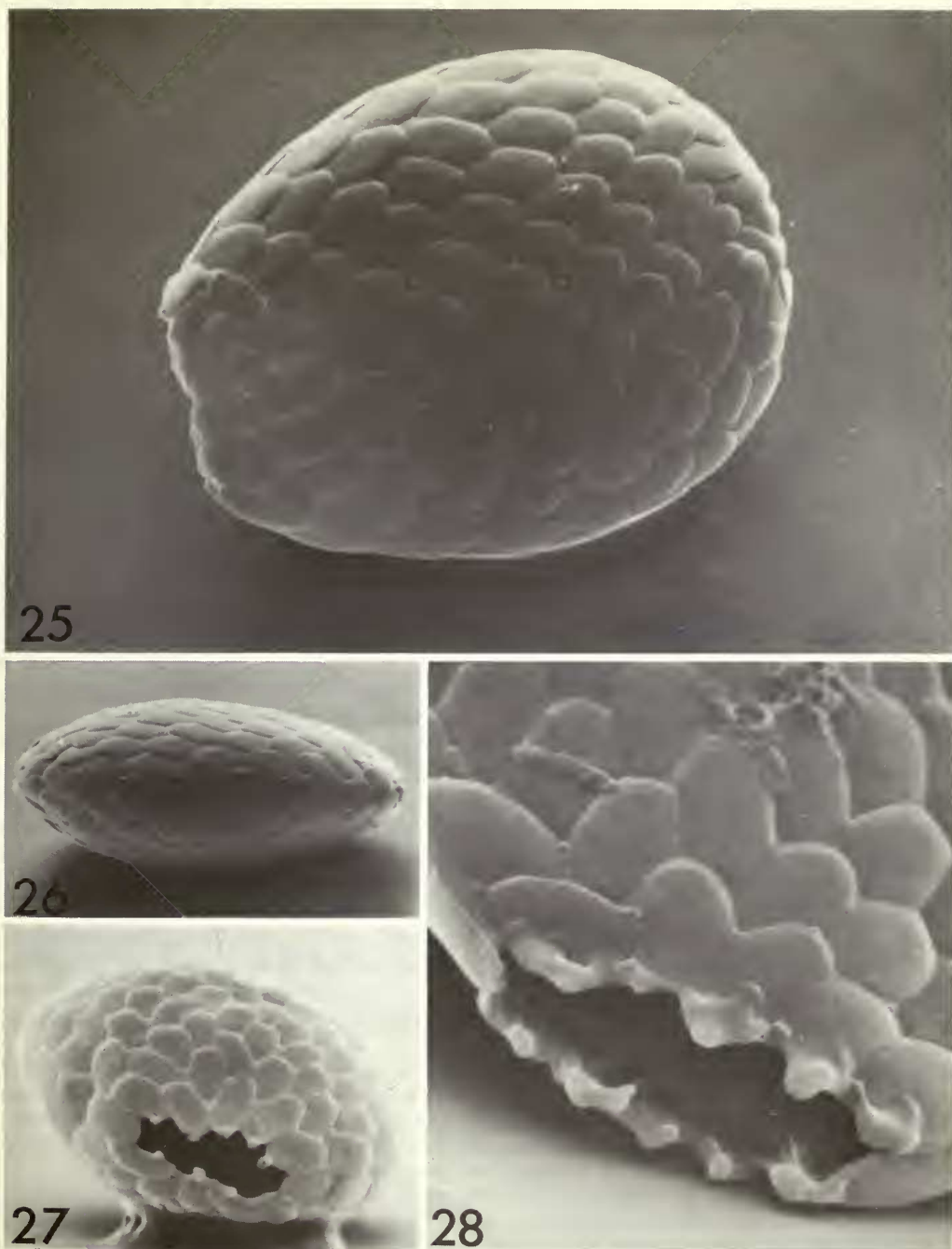
Figs 25–28 *Assulina muscorum*: Fig. 25, lateral view to illustrate the distribution of shell plates × 2150; Fig. 26, view to show lateral compression and arrangement of plates × 1400; Fig. 27, apertural view × 2100, Fig. 28, detail of 'tooth-like' projections of organic cement around the aperture × 4900.

longitudinal rows, with their major axis parallel to the major axis of the body. However, in some instances the axes of the plates are not parallel with the body and in these cases the general pattern is altered. The aboral extremity is also subject to irregular arrangements of shell plates.

DISCUSSION. The shell of *A. muscorum* is reported as being usually brown but occasionally colourless in the wild, whereas in culture it is mainly colourless. Nevertheless, live animals have a distinct band, probably the 'pigment zone', at the mid-body region which is often so large that it may tend to give colour to the shell. Variation in structure of the shell appears to be limited to the formation of an extra large individual, usually confined to less than three per cent of the population. Such specimens have more than the normal compliment of shell plates but the arrangement is the same. Similar large specimens have been reported (Hedley & Ogden, 1973) in clonal cultures of *Euglypha rotunda*.

Reproduction

The formation of a daughter-cell by simple division has been observed for all the described species and follows the same pattern in each, the only variation being the additional arrange-ment of elongated plates or spines in *E. acanthophora*, *E. cashii* and *E. compressa*. The sequence of events in *E. compressa* are described.

Euglypha compressa

The onset of division begins with the protrusion of a short thick cytoplasmic extension from the parent aperture. The apertural plates are the first to be passed from the storage area adjacent to the nucleus, via the peripheral cytoplasm to become arranged in a circle around this cytoplasmic extension. The remainder of the plates follow the same route and are arranged in sequence, in distinct rows radiating from the apertural plates (Fig. 29a). Each plate is added on the inside of the previous plate so that there is a considerable overlap. This excessive overlapping of the plates in the region of attachment is very noticeable in the early stages of shell construction. All the body plates are therefore in position, but not correctly spaced, well in advance of the shell attaining its full size. When the daughter shell has attained a size about two-thirds that of the parent, vacuolar activity is seen in the anterior third of the parental cytoplasm. This activity proceeds into the daughter cytoplasm as it increases in volume, at the same time the zone of pigment granules begin to extend towards the aperture of the parent. The spines are the last elements to be passed from the parent to the daughter (Figs 29b & c), where they are arranged centrally in the cytoplasm parallel to the shell walls. The pair of mid-body spines are the first to be pushed by cytoplasmic

Fig. 29   Diagram illustrating division in *Euglypha compressa*: formation of daughter shell (a–e) followed by nuclear division (f–l).
a. body plates (bp) pass via the peripheral cytoplasm to extremity of cytoplasmic extension of parent, 10–15 minutes; **b.** as last plates pass into forming daughter shell, siliceous spines (ss) begin moving from parent, 20 minutes; **c.** spines move into daughter shell, cytoplasmic vacuoles (v) appear close to parent aperture and granules of pigment zone (pz) begin to move, 25 minutes; **d.** spines arranged for positioning in daughter, and granules in parent move towards aperture, 27 minutes; **e.** daughter shell full size with some spines in position and cytoplasm filled with cytoplasmic vacuoles, granules begin to extend from parent into daughter, 30 minutes; **f.** daughter shell complete, granules in position between the opposed apertures and nucleus (n) in parent has small polar extensions, 40 minutes; **g.** elongation of parent nucleus, 55 minutes; **h.** granules mainly in daughter and nucleus now 'diamond-shape', 60 minutes; **i.** elongated nucleus becoming indistinct behind moving granules, 70 minutes; **j.** arrows depict pathways of rapidly moving granules, 75 minutes; **k.** movement of granules has slowed down and zones beginning to reform, faint nucleii appear, 90 minutes; **l.** contractile vacuoles (cv) and nucleii apparent, pigment zones almost reformed, 100 minutes. The times given are based on an estimated starting point.
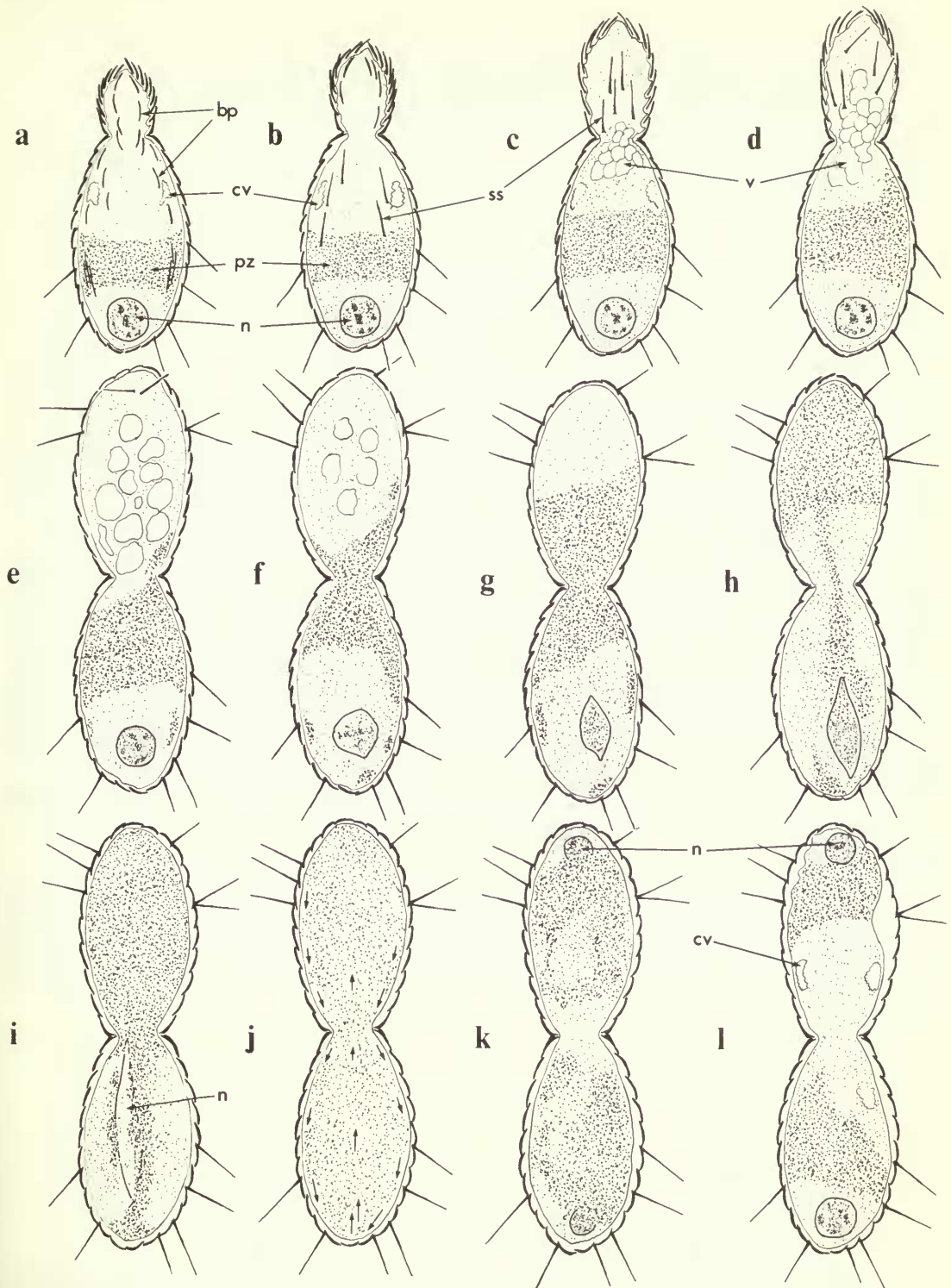
movement out between adjacent, lateral body plates, they appear to be positioned on each side simultaneously. This pair are followed in succession by other pairs of spines in a progressive sequence towards the aboral region. The last spines to be positioned are those that project from the terminal region. These spines are manoeuvred in the cytoplasm (Fig. 29e) until they are parallel to the aboral shell surface, they are then pushed through and cytoplasmic movement continues until the spines are at right angles to the shell surface. Throughout the time taken to position the spines those that project from the shell are in continuous movement, backwards and forwards like oarsmen in a rowing-boat except that their movements are not synchronized, and there are smaller movements still visible amongst the body plates. Vacuolar activity seems to be concentrated mainly in the daughter with the pigment granules being pushed from the parent into the peripheral cytoplasm of the daughter. A halo effect is most noticeable around the parent nucleus at this time.

A few seconds after the last aboral spines are in position, movement of all the shell elements slows down, until the spines are projecting slightly posteriorly when viewed from above. All movement has stopped after a further two minutes from the final positioning of the last aboral spines, and the daughter shell is complete. At about the same time the parent nucleus begins to elongate, initially there are two small polar extensions (Fig. 29f), but this changes into a 'diamond-shape' as most of the granules are passed into the daughter cytoplasm (Fig. 29g). Slightly later, movement of the granules in the daughter cytoplasm seem to suggest that when they reach the aboral extremity they are reflected back via the peripheral cytoplasm to the parent, meanwhile the nucleus has become more elongate (Fig. 29h). Rapid movement of the granules now obscures the changing nucleus (Fig. 29i), and a regular pathway of constantly moving granules is formed between the parent and daughter cytoplasm (Fig. 29j). When the rapid movement ceases, after about five minutes it is replaced by a slow regular movement with no apparent directional flow, cytoplasm of both cells looking homogenous. After a further five minutes the apertural region of both are relatively free of granules, contractile vacuoles are apparent in the anterior region of each and there is some movement of granules (Fig. 29k). A little while later nuclei are apparent in the posterior region of both cells as the pigment granules are concentrated into the mid-body region (Fig. 29l). Just prior to separation the cytoplasm in each shell is withdrawn slightly so that the cytoplasmic connection is severed, the animals move apart independently shortly after this action.

The approximate time taken to produce the daughter shell is forty-five minutes, whilst the total time for division is one hundred minutes.

Doubling time

Estimations on the length of time required to double the population (doubling time) were made on cultures established and maintained under similar conditions. Growth curves calculated from records of daily counts of individuals were made on three replicate cultures of four species, but only one culture was available for *E. acanthophora*. The results are given in Table 2.

Table 2   Estimates of doubling time (in days).

Euglypha acanthophora	2·7
Euglypha cashii	2·3–2·8
Euglypha compressa	4·0–4·4
Euglypha dickensii	2·6–3·1
Assulina muscorum	2·3–2·9

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